(11) (A) No. 974907

(45) ISSUED Sep. 23, 1975

(52) CLASS 195-71
C.R. CL.

(19) (CA) # CANADIAN PATENT

(54) ## PRODUCTION OF ENZYME PREPARATIONS

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(21) APPLICATION No. 118,142
(22) FILED July 13, 1971

(30) PRIORITY DATE July 28, 1970 (36564/70) G. B.

No. OF CLAIMS 11 - No drawing

## A B S T R A C T  O F  I N V E N T I O N

This invention relates to a process for the production of enzyme preparations consisting of uniformly sized solid spheres, which comprises subjecting enzyme-containing pellets prepared by extrusion from a mixture containing from 75 to 97 per cent of a solid enzyme-containing powder comprising, if desired, an enzyme stabilizer, and from 25 to 3 per cent of water to a spheronizing process using a rotational speed of up to about 2000 rpm in an apparatus causing centrifugal and frictional forces to be applied to the said pellets, whereafter, if desired, the solid spheres produced are subjected to a fluid-bed drying operation.

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A process for the production of enzyme preparations consisting of uniformly sized solid spheres, which comprises subjecting enzyme-containing pellets prepared by extrusion from a mixture containing from 75 to 97 per cent of a solid enzyme-containing powder and from 25 to 3 per cent of water to a spheronizing process using a rotational speed of up to about 2000 rpm in an apparatus causing centrifugal and frictional forces to be applied to the said pellets.

2. A process as claimed in claim 1, in which the solid enzyme-containing powder comprises an enzyme stabilizer.

3. A process as claimed in claims 1 or 2, wherein the solid spheres produced are thereafter subjected to a fluid bed drying operation.

4. A process as claimed in claim 1, in which the spheronizing process is carried out using a powdering agent preventing adhesion between the spheronized particles.

5. A process as claimed in claim 4, wherein the powdering agent is selected from the group consisting of an inorganic salt and an inorganic oxide.

6. A process as claimed in claim 1 or 4, in which the spheronizing process is carried out in a spheronizing apparatus having a rotational speed of up to about 2000 rpm, causing centrifugal and frictional forces to be applied to the material treated, said apparatus having a rotating friction plate moving in a plane forming an angle of 90° with stationary side walls.



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7. A process as claimed in claim 1, 2 or 4, in which the solid enzyme powder used comprises an enzyme stabilizer selected from the group consisting of gelatine, casein, skimmed milk powder and corresponding substrates for the enzymes used and polyvinylpyrrolidone.

8. A process as claimed in claim 1, 2 or 4, in which there is employed an enzyme powder wherein the enzyme is selected from the group consisting of proteases, amylases, amyloglucosidase and isomerases.

9. A process as claimed in claim 1, 2 or 4, in which there is employed an enzyme powder wherein the enzyme is selected from the group consisting of a protease from Bacillus licheniformis, an amylase from Bacillus subtilis, hemicellulase, fungal α-amylase and proteolytic enzymes prepared by aerobic cultivation of protease-forming species of the genus Bacillus on a nutrient medium having a pH within the range of 9 to 11 and maintaining during the main period of cultivation a pH in the said medium between 7.5 and 10.5, the said proteolytic enzymes showing a proteolytic activity of 80 to 100 per cent of maximum activity when measured at pH 12 by the Anson hemoglobin method carried out in the presence of urea.

10. A process as claimed in claim 1, 2 or 4, in which the enzyme-containing end product is coated in a manner known per se.

11. A process as claimed in claim 1, 2 or 4, in which said spheronizing process is at a rotational speed of 800 to 1000 rpm.



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This invention relates to a process for the production of enzyme preparations consisting of uniformly sized solid spheres.

In this specification and in the claims the expression "pellets" is intended to cover not only normal pellets, but also extruded, shaped bodies normally having an elongated structure, e.g. a spaghetti-like structure.

It is known to convert an extruded material into uniformly sized solid spheres by supplying the extruded pellets to a container with stationary solid side walls and a rotatably mounted bottom friction plate rotating with a speed from about 100 and up to 1800 rpm. This spheronizing is caused by centrifugal force and friction and has been performed in machines sold under the trademark MARUMERIZER® obtained from the Eli Lilly Company and manufactured by Fuji Denki Kogyo Company, Osaka, Japan.

We have now found that this spheronizing process is very useful in connection with enzyme preparations, particularly for use in the detergent industry, e.g. preparations comprising enzymes and additives normally used in washing and cleaning compositions, when the process is carried out with certain extruded enzyme-containing pellets. These pellets are produced in a conventional manner from a mixture of 75% to 97% of a solid enzyme-containing powder and 25% to 3% of water.

According to the invention there is provided a process for the production of enzyme preparations consisting of uniformly sized solid spheres, which comprises subjecting enzyme-containing pellets prepared by extrusion from a mixture containing from 75% to 97% of a solid enzyme-containing powder and from 25% to 3% of water to a spheronizing process using a rotational speed of up to about 2000 rpm in an apparatus causing centrifugal and frictional forces to be applied to the said pellets.

According to one aspect of the invention the solid spheres produced are subjected to a fluid-bed drying operation.

The enzyme preparations which can be produced by the pro-

cess of this invention consist of particles of practically uniform size suitable for the intended industrial uses. The particles are substantially dust-free and show a sufficient mechanical strength for handling without the formation of dust. The particles also show sufficient flow properties for transportation in factories.

In the following examples rotational speeds of up to about 800-1000 rpm are used during the spheronization, but speeds up to about 2000 rpm may be employed.

In accordance with a preferred embodiment of the invention the spheronizing process is carried out in a machine of the type marketed under the trademark NARUMERIZER ® referred to above.

The product prepared by the process of the invention is easily soluble in hot as well as cold water. This is of special advantage when an enzyme product is to be used as an additive to a prewashing agent or a soaking agent.

The products of the present process possess a good storage stability, even under unfavourable conditions as regards temperature and humidity, and also when these products are used in perborate-containing washing agents.

If desired, the products prepared in accordance with the invention may be further improved by coating in a manner known per se with a tablet coating composition, e.g. as described in J.Am.Pharm. Association, Aug. 1954, Vol. XLIII, No. 8. Preferably the coating is carried out using a waxy substance, if desired a slightly sticky substance, but the coating agent should be easily soluble or dispersable in water.

Examples of preferred coating materials are as mentioned in the above literature polyethyleneglycol 6000 through 1000, but also nonylphenol-polyglycol-ethers having from 16 to 50 ethyleneglycol units, ethoxylated fatty alcohols in which the hydrocarbon moiety of the alcohol contains from 12 to 20 carbon atoms and the polyglycol moiety comprises from 15 to 80 polyethyleneglycol units.

fatty alcohols, fatty acids and mono- and diesters of fatty acids and glycerol.

The optional coating process of the invention may be carried out in a simple and inexpensive apparatus, such as a mixing apparatus of the drum type having rotatable mixing aggregates. Thus, the use of complicated and expensive special kettles or fluidizing units comprising nozzle arrangements can be avoided. Furthermore, it is often possible merely to melt the coating material and pour or spray it into the mixing drum, thus avoiding special solution processes.

The coated products are suitable for colouring with e.g. titanium dioxide or pigment colours, and the coated products are also properly protected against possible abrasion giving rise to the formation of undesirable enzyme-containing dust.

The enzyme-containing powder in addition to the enzyme itself preferably contains suitable additives, such as lubricants, fillers, binders and enzyme stabilizers. Polyethyleneglycols are examples of suitable lubricants, and examples of fillers are inorganic salts, for instance sodium chloride and sodium sulfate, pentasodiumtripolyphosphate, tetrasodiumpyrophosphate or the corresponding potassium salts, cellulose powder, starch powder, cellulose derivatives, starch decomposition products, starch derivatives, gelatine, casein, skimmed milk powder, polyvinylalcohol and polyvinyl-pyrrolidones. Some of these substances may also act as binders. This applies for instance to the starch decomposition product dextrin, polyvinylpyrrolidone and polyvinylalcohol. Gelatine, starch decomposition products, and other substrates for the enzymes and polyvinylpyrrolidone are examples of enzyme stabilizers. In particular, casein, skimmed milk powder and

polyvinylpyrrolidone have been found to be useful.

Furthermore, polyvinylpyrrolidone acts in such a manner that each single string of extrudate becomes less adhesive so that the tendency to string adhesion in the spheronizing process is lowered.

In the spheronizing steps it can be advantageous to use a powdering agent to prevent any tendency of adherence between the spheronized particles. Examples of such powdering agent are inorganic salts, such as anhydrous sodium sulfate, and inorganic oxides such as titanium dioxide.

The ratio between the enzyme powder and water in the mixture to be spheronized depends on the enzymatic activity of the enzyme powder and the desired enzymatic activity of the final spheronized enzyme product.

The following examples illustrate the process of the invention. In some of these examples we have used an enzyme concentrate called ALCALASE® (trademark), which is a commercial product and contains a proteolytic enzyme together with some inactive organic matter and some inorganic salts, mainly sodium sulfate. In an example, we have also used an enzyme concentrate called TERMOZYM® (trademark) which is a commercial product and contains an amylolytic enzyme together with some inactive organic matter and some inorganic salts, mainly sodium sulfate.

Furthermore, the working examples comprise examples showing the use of hemicellulase, fungal α-amylase as well as a proteolytic enzyme called ENZYME X and produced as described in copending Canadian application Serial No. 030,578 filed September 20, 1968 - K. Aunstrup, O. Andresen and N. Nattrup, by cultivation of the Bacillus strain NCIB No. 10147 (NCIB No. 10147 is a deposit number for the said strain at the National Collection of Industrial Bacteria, Torry Research Station, Aber-

dam, Scotland). Apart from the SUBTILISIN A there may be used similar proteolytic enzymes prepared by aerobic cultivation of protease-forming species of the genus Bacillus on a nutrient medium having a pH within the range of 9 to 11 and containing during the main period of cultivation a pH in the said medium between 7.5 and 10.5, the said proteolytic enzymes showing a proteolytic activity of 80 to 100 per cent of maximum activity when measured at pH 12 by the Anson hemoglobin method carried out in the presence of urea. Furthermore, other amylases and proteinases, as well as milk-coagulating enzymes, cellulases, glucoseisomerase, pectinases, amyloglucosidase and β-glucanase may be employed.

The percentages in the examples are per cent by weight.

Example 1

There is produced a premix consisting of 30% ALCALASE® and 70% sodium sulfate, and this mixture is moistened in a mixing aggregate with 6% of water which is sprayed on the mixture.

The moistened mixture is extruded in the conventional manner through a 0.7 mm screen, and the pellets formed are then spheronized in a MARUMERIZER® at a beginning speed of 400 rpm while powdering with 3% of titanium dioxide and finally at a speed of 800 rpm. Any traces of dust from the powdering substance can be removed by screening.

The final product has the following properties:

| | |
|---|---|
| Proteolytic activity | 1.3 Anson units/g |
| Particle size | 0.7 mm |
| Bulk weight | about 1.0 g/cm$^3$ |

The product is dust-free and soluble in aqueous media.

A premix consisting of 90% ALCALASE® and 10% sodium chloride is mixed with 6% of water and extruded and spheronized as described in Example 1. The final product has the same properties as those mentioned in connection with the final product produced in Example 1.

Example 3

A premix having the following composition:

| | |
|---|---|
| 25% | ALCALASE® |
| 10% | Dextrin |
| 5% | Cellulose powder |
| 6% | Polyethyleneglycol 6000 |
| 54% | Anhydrous sodium sulfate |

is moistened with 8% of water, and the moistened mixture is extruded in the conventional manner through a 0.8 mm screen. The pellets formed are spheronized as in Example 1, except that anhydrous sodium sulfate is used as powdering agent instead of titanium dioxide.

The final product has the following properties:

| | |
|---|---|
| Proteolytic activity | 1.0 Anson units/g |
| Particle size | 0.8 mm |
| Bulk weight | about 1.0 g/cm$^3$ |

Example 4

A premix consisting of 25% ALCALASE®, 10% cellulose powder and 65% sodium sulfate is moistened with 17.5% of an aqueous solution containing 10% hydroxypropyl-cellulose and 2% polyethyleneglycol 6000.

The moistened mixture is extruded and spheronized as in Example 1, and the final product has the same properties as those mentioned in connection with the final product produced in Example 3.

Hydroxypropylcellulose may be substituted by polyvinylpyrrolidone.

Example 5

A mixture consisting of 33.5% ALCALASE ®, 25% TERMOZYM ®, 18% dextrin, 18.5% cellulose powder and 5% polyethyleneglycol 6000 is moistened with 16% of water and extruded in the conventional manner through a 0.8 mm screen. The pellets formed are then spheronized as described in Example 1.

The final product has the following properties:

| | |
|---|---|
| Proteolytic activity | 1.3 Anson units/g |
| Amylolytic activity | 135 SKB units/g |
| Particle size | 0.8 mm |
| Bulk weight | 0.9 g/cm$^3$ |

Example 6

A powder mixture of the composition:

| | |
|---|---|
| 33% | ALCALASE ® |
| 10% | Cellulose powder |
| 3% | Gelatine |
| 60% | Anhydrous sodium sulfate |
| 2% | Polyethyleneglycol 6000 |

is moistened with 16% of water and is extruded and spheronized as described in Example 3. The final product has the same properties as those mentioned in connection with the product produced in Example 3.

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Example 7

A powder mixture of the composition

6.4% ALCALASE (R)

10.4% Skimmed milk powder

82.6% Sodium chloride

was moistened with 18.5% of a solution of the composition

5% Water

35% Polyethyleneglycol 6000

12% Polyvinylpyrrolidone

The wetted mixture was extruded and spheronized as described in Example 3.

The spheronized product was fluid-bed dried at 40 to 50°C to a moisture content of about 0.5%.

The final product has following properties:

| | |
|---|---|
| Proteolytic activity | 0.3 AU/g |
| Particle size | 0.7 mm |
| Bulk density | 1.05 g/cm$^3$ |
| Soluble in water | |

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Example 8

Powder mixtures of the composition

5.5% ALCALASE®

5.5% or 11% Casein

89% or 84.5% Sodium chloride

were moistened with 16.5% of a solution of the composition

55% Water

35% Polyethyleneglycol 6000

12% Polyvinylpyrrolidone

The wet mixture was extruded and treated as described in Example 5.

The properties of the final product are as described in Example 7.

Example 9

A premix of the composition

3.0% ENZYME X (prepared from strain NCIB No. 10147)

2.0% Polyvinylpyrrolidone

6.0% Polyethyleneglycol (6000)

89% Sodium chloride

was moistened with 8% of water and extruded through a 0.9 mm screen and spheronized at a speed of 1000 rpm.

The wet product was fluid-bed dried to a moisture content of approximately 0.5%.

The properties of the final product were

| | |
|---|---|
| Proteolytic activity | 1 KNPU/g |
| Particle size | appr. 0.8 mm |
| Bulk density | appr. 1.1 g/cm$^3$ |
| Water soluble | |

A premix of the composition

12% ALCALASE ®

5% Polyethyleneglycol 6000

1% Polyvinylpyrrolidone

84% Sodium citrate

was moistened with 9.5% of water and extruded, spheronized and dried as described in Example 9.

The properties of the final product are

| | |
|---|---|
| Proteolytic activity | 0.5 AU/g |
| Particle size | 0.7 mm |
| Water soluble | |

Example 11

A premix of the composition

2% Hemicellulase

6% Polyethyleneglycol 6000

2% Polyvinylpyrrolidone

90% Glucose

was moistened with 7% of water and extruded through a 0.9 mm screen and spheronized at 900 rpm.

The wet product was fluid-bed dried at 40°C to a moisture content below 1%.

The properties of the final product were

| | |
|---|---|
| Enzymatic activity | 50,000 VHCE/g |
| Particle size | 0.9 mm |
| Bulk density | 0.8 g/cm$^3$ |
| Water soluble | |

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Example 12

A premix of the composition

3% Fungal α-amylase

67% Sodium chloride

2% Polyvinylpyrrolidone

is moistened with 12% of water and extruded through a 0.8 mm screen and spheronized at a speed of 800 rpm.

The spheronized product was fluid-bed dried at 50°C, and the final product had the following properties

| | |
|---|---|
| Enzymatic activity | 3000 FAU/g |
| Particle size | 0.7 mm |
| Bulk weight | about 0.9 g/cm$^3$ |

Example 13

A premix of the composition

26% ALCALASE®

6% Pluronic L 61

70% Sodium tripolyphosphate (Marchon type d)

was moistened with 12.5% of water and extruded through a 0.9 mm screen.

The extrudate was spheronized as described in Example 3.

The final product had the following properties

| | |
|---|---|
| Proteolytic activity | 1.0 AU/g |
| Particle size | 0.9 mm |
| Bulk density | approx. 1 g/cm$^3$ |
| Soluble in water | |

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Example 14

A premix of the composition

| | |
|---|---|
| Bacterial amylase | 10% |
| Polyethyleneglycol 6000 | 6% |
| Polyvinylpyrrolidone | 2% |
| Sodium chloride | 77% |

was moistened with 5% of water and extruded through a 0.9 mm screen. The extrudate was broken in the MARUMERIZER (R) to form "noddles" each having a length of 1 to 3 mm.

Enzymatic activity 250 KNU/g

Particle size: Small cylinders having rounded-off end faces: 0.8 mm x 1-3 mm

Bulk density about 1 g/cm$^3$

The granulate was dried in fluid-bed at temperatures 40°C -> 60°C.

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When the enzyme preparations prepared by the present process are intended for washing purposes, experiments made demonstrate that the storage stability in washing agents is satisfactory, in particular when the enzyme stabilizers referred to in the foregoing are used:

Storage stability in perborate-containing washing agent prepared on the basis of

Residual activity 30°C; 70% rel. moisture Method of analysis: TNBS

| | 2 weeks | 4 weeks | 6 weeks | 8 weeks |
|---|---|---|---|---|
| Example 7 (10.9% skimmed milk powder) | 92% | 74% | 63% | 62% |
| References: 6.5% ALCALASE® 2% PVP 6% PEG 6000 86% NaCl | 84% | 60% | 52% | 49% |

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Storage stability in perborate-containing washing agent

Residual activity
35°C; 67% rel. moisture
Method of analysis:
TAED

| | 2 weeks | 4 weeks | 6 weeks | 8 weeks |
|---|---|---|---|---|
| AUCALASE®, powdered, ungranulated (double test) | 30% | 14% | 13% | --- |
| Reference, granulated (double test) | 53% | 24% | 25% | --- |
| Granulate 2% PVP (double test) | 52% | 38% | 36% | --- |
| Granulate 4% PVP (double test) | 58% | 44% | 41% | --- |
| Granulate 7% PVP (4 tests) | 45% | 41% | 34% | 30% |
| Ref. (4 tests) | 36% | 18% | 18% | 11% |

| | Residual Activity 35°C; 67% rel. moisture Method of analysis: TNBS | | | |
|---|---|---|---|---|
| | 1 week | 2 weeks | 4 weeks | 6 weeks |
| Example 7 | 93% | 70% | 65% | --- |
| Ref. to Example 7 | 73% | 65% | 47% | --- |
| Example 8 (11% Casein) | --- | 75% | 64% | 51% |
| Example 8 (55% Casein) | --- | 72% | 54% | 43% |
| Granulate 2.5% skimmed milk powder | --- | 46% | 10% | --- |
| Granulate 5% skimmed milk powder | --- | 52% | 42% | --- |
| Reference | --- | 29% | 24% | --- |

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| Storage stability in perborate-containing washing agent | Residual Activity 30°C; 70% rel. moisture | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 week | 2 weeks | 3 weeks | 4 weeks | 5 weeks | 6 weeks | 7 weeks | 8 weeks |
| A. Granulate with 10% casein | 91% | 94% | 94% | 68% | 61% | 58% | --- | --- |
| B. Ref. to A | 91% | 80% | 70% | 44% | 29% | 24% | --- | --- |
| C. Granulate with 5% casein | 100% | --- | --- | 85% | --- | --- | 58% | 47% |
| D. Ref. to C | 91% | --- | --- | 70% | --- | --- | 29% | 25% |

In the foregoing, the proteolytic activities have been determined by the Anson-method described in J.Gen.Physiol, 22, 79-89 (1938). The TNBS-method for determining protease activity is described in J.Am.Oil Chem. Soc., 46:81 (1969). α-amylase activities have been determined according to Cereal Chemistry 16, 712 (1939), but with some modifications; thus, the following equations can be used for calculations:

1000 SKB units (pH 5.7) ~ 53000 NOVO units for bacterial α-amylase and

1000 SKB units (pH 4.7) ~ 57 FA units for fungal α-amylase

Hemicellulase activity has been determined viscosimetrically.

# SUBSTITUTE

## *REMPLACEMENT*

## SECTION is not Present

## *Cette Section est Absente*